

What is claimed is:

1. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an isoleucine residue in a first position corresponding to position 132 of SEQ ID NO: 4.
2. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, wherein a non-natural amino acid is incorporated into a position corresponding to 132 of SEQ ID NO: 4, during translation of said protein.
3. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 69 of SEQ ID NO: 4.
4. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 70 of SEQ ID NO: 4.
5. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 74 of SEQ ID NO: 4.
6. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 76 of SEQ ID NO: 4.

7. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a phenylalanine residue in a first 5 position corresponding to position 132 of SEQ ID NO: 4.

8. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an tyrosine residue in a first 10 position corresponding to position 86 of SEQ ID NO: 4.

9. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first 15 position corresponding to position 66 of SEQ ID NO: 4.

10. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first 20 position corresponding to position 65 of SEQ ID NO: 4.

11. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a tyrosine residue in a first 25 position corresponding to position 16 of SEQ ID NO: 4.

12. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a tryptophan residue in a first 30 position corresponding to position 82 of SEQ ID NO: 4.

13. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a phenylalanine residue in a first position corresponding to position 82 of SEQ ID NO: 4.

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14. A kit comprising the protein of claim 1 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

15. A kit comprising the protein of claim 2 and a coelenterazine selected from the 10 group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

16. A kit comprising the protein of claim 3 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

15 17. A kit comprising the protein of claim 4 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

18. A kit comprising the protein of claim 5 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

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19. A kit comprising the protein of claim 6 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

25 20. A kit comprising the protein of claim 7 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

21. A kit comprising the protein of claim 8 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

22. A kit comprising the protein of claim 9 and a coelenterazine selected from the 30 group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

23. A kit comprising the protein of claim 1 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

24. A kit comprising the protein of claim 10 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

25. A kit comprising the protein of claim 11 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

10 26. A kit comprising the protein of claim 12 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

27. A kit comprising the protein of claim 13 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

15 28. An aequorin mutant protein encoded by the nucleic acid of claim 3 wherein the protein is conjugated to a fluorophore.

29. The aequorin mutant of claim 28 wherein the fluorophore is IANBD ester.

20 30. An aequorin mutant protein encoded by the nucleic acid of claim 4 wherein the protein is conjugated to a fluorophore.

31. The aequorin mutant of claim 30 wherein the fluorophore is IANBD ester.

25 32. An aequorin mutant protein encoded by the nucleic acid of claim 5 wherein the protein is conjugated to a fluorophore.

33. The aequorin mutant of claim 32 wherein the fluorophore is IANBD ester.

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34. An aequorin mutant protein encoded by the nucleic acid of claim 6 wherein the protein is conjugated to a fluorophore.

35. The aequorin mutant of claim 34 wherein the fluorophore is IANBD ester.

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36. The nucleic acid of claim 2 wherein the non natural amino acid is fluorotyrosine or fluorotryptophan.

37. The nucleic acid of claim 36 wherein the fluorotyrosine is 3-fluoro-l-tyrosine.

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38. The nucleic acid of claim 2 wherein the non natural amino is 5-fluoro-l-tryptophan.

39. A method of identifying inhibitors of bond-breaking enzymes comprising:

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(a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:

(1) any one of the nucleic acids of claims 1 to 13;

(2) operably linked to a second nucleic acid encoding a bond-breaking enzyme recognition site;

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in a first locus and a second locus;

(b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;

(c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and

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(d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

40. A method of identifying inhibitors of HIV-1 protease comprising:

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(a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:

(1) an isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having one or two amino acid substitutions selected from the group consisting of, an isoleucine residue in a position corresponding to position 132 of SEQ ID NO: 4, a non-natural amino acid incorporated into a position corresponding to 132 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 69 of SEQ ID NO: 4; a cysteine residue in a position corresponding to position 70 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 74 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 76 of SEQ ID NO: 4, a phenylalanine residue in a position corresponding to position 132 of SEQ ID NO: 4, a tyrosine residue in a position corresponding to position 86 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 66 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 65 of SEQ ID NO: 4, a tyrosine residue in a position corresponding to position 16 of SEQ ID NO: 4, a tryptophan residue in a position corresponding to position 82 of SEQ ID NO: 4, and a phenylalanine residue in a position corresponding to position 82 of SEQ ID NO: 4;

5 (2) operably linked to a second nucleic acid encoding an HIV-1 enzyme recognition site;

10 in a first locus and a second locus;

15 (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;

20 (c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and

25 (d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

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41. The method of claim 40 wherein the recognition site is Ser-Glu-Asn-Tyr-Pro-Ile-
Val (SEQ ID NO: 5).

5 42. The method of claim 40 wherein the fusion protein is conjugated to a fluorophore.

43. The method of claim 40 wherein the fusion protein comprises a non-natural amino
acid.

10 44. The method of claim 43 wherein the non-natural amino acid is fluorotyrosine and
is at a position corresponding to 132 of SEQ ID NO: 4.

45. The method of claim 40 wherein the nucleic acid capable of hybridizing to SEQ
ID NO: 3 under stringent conditions is any of the nucleic acids recited in claims 1
15 to 13.

46. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 5 under stringent
conditions and encoding a protein which is capable of binding coelenterazine and
molecular oxygen and emitting light, said protein having an serine residue in a first
20 position corresponding to position 51, and a serine residue in a second position
corresponding to position 75 of SEQ ID NO: 6.

47. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 5 under stringent
conditions and encoding a protein which is capable of binding coelenterazine and
25 molecular oxygen and emitting light, said protein having an serine residue in a first
position corresponding to position 67, and a serine residue in a second position
corresponding to position 75 of SEQ ID NO: 6.

48. An isolated nucleic acid capable of hybridizing to SEQ ID NO: 5 under stringent
30 conditions and encoding a protein which is capable of binding coelenterazine and

molecular oxygen and emitting light, said protein having an serine residue in a first position corresponding to position 151 of SEQ ID NO: 6.

49. A kit comprising the protein of claim 46 and a coelenterazine selected from the

5 group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

50. A kit comprising the protein of claim 47 and a coelenterazine selected from the

group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

10 51. A kit comprising the protein of claim 48 and a coelenterazine selected from the

group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

52. A method of identifying inhibitors of bond-breaking enzymes comprising:

(a) immobilizing a fusion protein encoded by a fusion protein nucleic acid
15 comprising:

- (1) any one of the nucleic acids of claims 46 to 48;
- (2) operably linked to a second nucleic acid encoding a bond-breaking enzyme recognition site;

in a first locus and a second locus;

20 (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;

(c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and

25 (d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

53. A method of identifying inhibitors of HIV-1 protease comprising:

(a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:

- (1) any one of the nucleic acids of claims 46 to 48;

(2) operably linked to a second nucleic acid encoding an HIV-1 enzyme recognition site;

in a first locus and a second locus;

5 (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;

(c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and

(d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

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